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During the period (1993-1997) of support from AFOSR, we completed many papers. Some of those listed below deal exclusively with the subject of the proposal, the suprachiasmatic nucleus, and some deal indirectly with issues related to the circadian clock or transmitters found in the SCN. A substantial effort was invested in examining the two primary transmitters in the SCN, glutamate, which is excitatory, and GABA, which is inhibitory. These two are particularly important because the primary input from the retina that phase-shifts the clock is glutamate, and the primary transmitter made by SCN cells themselves is GABA.

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Final report: July 1997

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AFOSR- Chronobiology Program Award # F49620-93-1-0283-PO2
"Cellular interactions in the circadian clock- the suprachiasmatic nucleus"
Air Force Program Manager: Dr.Genevieve Haddad

This is the final report on our research grant from the AFOSR to Stanford University. May 1997 marked the end of a 1 year no-cost time extension that allowed us to complete work on our project that had been started during the previous three years. This one year extension was very helpful, and because of this we were able to publish several additional papers on the circadian clock in the hypothalamus, the suprachiasmatic nucleus (SCN).

Major Findings.

During the period (1993-1997 of support from AFOSR, we completed many papers. Some of those listed below deal exclusively with the subject of the proposal, the suprachiasmatic nucleus, and some deal indirectly with issues related to the circadian clock or transmitters found in the SCN.

A substantial effort was invested in examining the two primary transmitters in the SCN, glutamate, which is excitatory, and GABA, which is inhibitory. These two are particularly important because the primary input from the retina that phase-shifts the clock is glutamate, and the primary transmitter made by SCN cells themselves is GABA.

Immunocytochemistry, electrophysiology, digital calcium imaging, and molecular biology was used to study GABA and glutamate in the SCN, and to study how the other transmitters found in the SCN can modulate release or response to these two primary transmitters. One important finding was that when glutamate and GABA receptors were blocked with specific antagonists, no postsynaptic potentials could be found either in SCN slices or in SCN cultures. This suggests that in the absence of glutamate and GABA, no other transmitter is by itself shows much synaptic activity in the SCN.

Glutamate plays a significant role in initiating phase shifts of circadian rhythms. Recent evidence suggests that both metabotropic and ionotropic glutamate receptors may influence phase shifts. With electron microscopy, immunoreactivity for different metabotropic GluRs was found both pre- and postsynaptically in neurons and in astrocytes. Using cDNA-PCR and immunocytochemistry, we found that mRNA for all 8 metabotropic GluR genes that have been cloned to date is expressed by SCN neurons, including a new splice variant, mGluR7b. With whole cell patch clamp recording using a novel model of single self-innervating neurons, all three class of mGluRs can be found in single SCN axons, and these presynaptic mGluRs modulate each other.

Neuropeptide Y (NPY) is found in SCN innervation arising from the thalamic intergeniculate leaflet, a visual relay pathway. Injections of NPY into the SCN cause phase shifts in behavior and electrical activity. Although in many other regions of the brain such as the hippocampus NPY has little effect on GABAergic neurons, in the SCN a substantial depression of GABA release was found. NPY reduced the amplitude and frequency of inhibitory postsynaptic

currents (IPSCs) in SCN neurons, acting at Y1- and Y2-type receptors coexpressed in single SCN axons. In the presence of tetrodotoxin, SCN axons still released GABA independent of action potentials. NPY reduced the frequency of miniature IPSCs in the presence of tetrodotoxin, supporting a presynaptic mechanism of action. NPY also reduced the frequency and amplitude of glutamate-mediated excitatory postsynaptic currents in SCN slices. In developing SCN neurons, NPY reduced GABA-mediated excitation and calcium rises, in part by a mechanism that may involve protein kinase A and cAMP modulation.

One interesting and novel outcome of our data is that many of the transmitters of the SCN exert their most striking effect not on the cell body, but rather on the axon terminal. In most cases, the effect on the axon terminal was to reduce release of the axon's transmitter. The mechanism for this was sometimes based on reducing the inflow of calcium ions, a necessary prerequisite for transmitter release. Previous work on the SCN has focused on transmitter effects on the neuron cell body. Our work supported by the Air Force is the first to find large effects of SCN transmitters that act on axon terminals of the SCN neurons.

Relevance to the Air Force mission.

A significant problem to the Air Force is how to maintain human performance at peak efficiency at times of the 24 hour day when performance is usually low, for instance in the middle of the night. It has been estimated that 65% of accidents involving aircraft may be due to human error, and human error is most likely at times of the day when sleep is usually occurring. Part of this is due to the circadian clock in the brain that regulates states of sleep and wakefulness. Our work at the cellular level focuses on the mechanisms by which the internal clock in the suprachiasmatic nucleus can respond to naturally occurring substances in the brain that underlie normal phase shifts. The next step will be to ask questions about how this information can be used and adapted to provide potential chemicals that may aid in phase shifting the human biological clock, which would be of great benefit to enhance performance without the side effects of some of the drugs that currently are used, for instance amphetamines.

This work is relevant to potential mechanisms of phase shifting of circadian rhythms. An intimate understanding of how single SCN neurons respond to agents that underlie phase shifting is leading to a better knowledge of how to accelerate phase shifting of the human circadian clock, which would ultimately lead to an enhancement of performance under conditions of day-night shifts in work schedules and jet-lag. For instance, NPY can phase shift the circadian clock substantially. Our data on NPY suggest that it can reduce transmitter release, acting by reducing calcium influx in the terminal. This suggests that one could potentially use a chemical similar to NPY to phase shift circadian rhythms of pilots or flight surgeons who are involved in sustained operations over several days. Whereas NPY by itself does not cross the blood brain barrier, other newer NPY-like substances have just become available that do cross the blood brain barrier, and would be able to get to the SCN. These substances should be available in the near future, and may be of significant use in chemical facilitation of phase shifting to enhance performance at times when humans would normally be sleeping, and quite possibly without the side effects of drugs now be used. Alternately, drugs that act directly on calcium channels (like NPY does) may have the same effect as NPY, and would provide another route of drug discovery for phase shifting the endogenous clock.

During this period of support, the following scientists worked on the experiments.

Anthony N. van den Pol,PhD
Andrei Belousov, PhD
Gong Chen, PhD
Vinh Cao

Karl Obrietan, PhD
Paul Franken, PhD
Roger Morrisette
Laure Haak, PhD

Publications. Full length papers only.

The following papers done between 1994 and 1997 acknowledged AFOSR support. Some focus on the SCN (in bold), and others examine related topics, but include information relevant to the SCN or its neurotransmitters.

van den Pol, A.N., and Trombley, P.Q. (1993) Glutamate neurons in hypothalamus regulate excitatory transmission. Journal of Neuroscience 13: 2829-2836.

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Romano, C., M.A. Sesma, C. MacDonald, K. O'Malley, A.N. van den Pol and J. Olney (1995) Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. J. Comp. Neurology. 355: 455-469.

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Romano,C., A.N.van den Pol, and K.L.O'Malley (1996) Enhanced early developmental expression of the metabotropic glutamate receptor, mGluR5 in rat brain: protien, mRNA splice variants, and regional distribution.Journal of Comparative Neurology, 367:403-412.

Obrietan,K., and A.N.van den Pol (1996) NPY depresses GABA-mediated calcium transients in developing suprachiasmatic nucleus neurons: a novel form of calcium long term depression. J. Neurosci.16: 3521-3533.

van den Pol,A.N., G.J.Strecker, F.E.Dudek (1996) Excitatory and inhibitory amino acids and synaptic transmission in the suprachiasmatic nucleus. Progress in Brain Research. 111: 41-56.

van den Pol A.N., A.Belousov, K.Obrietan (1996) Glutamate hyperexcitability and seizure-like activity throughout brain and spinal cord upon relief from glutamate receptor blockade in vitro. Neurosci.,74: 653-674.

Chen,G., P.Trombley, and A.N.van den Pol (1996) Excitatory actions of GABA in developing neurons. Journal of Physiology (Lond.) 494: 451-464.

van den Pol,A.N., Obrietan K, Chen G (1996) Excitatory effects of GABA after neuronal trauma. Journal of Neuroscience. 16: 4283-4292.

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van den Pol,A.N, V.Cao, and A.Belousov (1996) Dopamine modulation of glutamate-regulated calcium and electrical activity in hypothalamic neurons. Journal of Neurophysiology, 76: 3934-3948.

van den Pol,A.N., K.Obrietan, G.Chen, and A.Belousov (1996) NPY mediated long term depression of excitatory activity in suprachiasmatic nucleus neurons. Journal of Neuroscience, 16: 5883-5895.

Chen,G. and A.N. van den Pol (1996) NPY Y1- and Y2-like receptors coexist in pre- and postsynaptic sites: inhibition of GABA release in isolated self-innervating SCN neurons. Journal of Neuroscience 16: 7711-7724.

Haak,L., H.C.Heller, and A.N.van den Pol (1997) Metabotropic glutamate receptor activation modulates kainate and serotonin calcium

response in astrocytes. Journal of Neuroscience 17: 1825-1837. (studied in suprachiasmatic nucleus astrocytes)

Belousov,A., and A.N.van den Pol (1997) Local synaptic release of glutamate from neurons of the rat hypothalamic arcuate nucleus. Journal of Physiology (Lond.), 499: 747-761.

van den Pol,A.N. (1997) GABA immunoreactivity in hypothalamic neurons and growth cones in early development in vitro prior to synapse formation. Journal of Comparative Neurology, In press.

Chen,G. and A.N.van den Pol (1997) Adenosine modulation of calcium currents and presynaptic inhibition of GABA release in suprachiasmatic and arcuate nucleus neurons. J.Neurophysiol., In press.

Ghosh,P.K., N.Baskaran, and A.N.van den Pol (1997) Developmentally regulated gene expression of all eight metabotropic glutamate receptors in hypothalamic SCN and arcuate nuclei-. Develop.Brain Res. In press.

+ 18 abstracts (not listed)

The papers above cited AFOSR support. Most are relevant to the SCN.

Lectures.
During the course of this application, the PI was invited to give lectures on the topic of the results of this grant at the following places:

Dartmouth College, New Hampshire
Gordon Conference, Vermont
Netherlands Brain Research Institute, Amsterdam, Holland
Yale University, Connecticut
Smith College, Mass.
Stanford University, Calif.
Colorado State Univ. Fort Collins, Colo.
Data was also presented at the national meeting of the Society for Neuroscience in
Miami, Florida
Washington, DC
San Diego, Calif.

No patents were filed (1993-1997).